Journal of Strength and Conditioning Research Publish Ahead of Print
DOI: 10.1519/JSC.0000000000001108

The Physiological Effects of Nucleotide Supplementation on Resistance Exercise Stress in Men and Women

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Running Head: Nucleotide Supplementation and Exercise

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Abstract

Nucleotide supplementation has been shown to attenuate post-exercise immunosuppression and HPA axis activation in endurance exercise models. These findings indicated that nucleotide supplementation may aid in the recovery from other exercise modalities, such as heavy resistance exercise. Thus, the purpose of this investigation is to investigate the effects of nucleotide supplementation on the acute cortisol and immune system responses to heavy resistance exercise and its effects on recovery. A double blinded, cross over, within subject design, with ten men and ten women was used. Each performed an acute heavy resistance exercise protocol (AHREP) following a loading period with either a nucleotide supplement or placebo supplementation phase. Before and after the AHREP, and at 24, 48, and 72 hrs thereafter, blood samples were analyzed for cortisol, myeloperoxidase, and absolute neutrophil, lymphocyte and monocyte counts. Creatine kinase was analyzed pre-AHREP and at 24, 48, and 72 hrs post-AHREP. Performance measures, including peak back squat isometric force and peak countermovement jump power were also analyzed. Nucleotide supplementation resulted in significant ($P \leq 0.05$) decreases in observed cortisol and MPO acutely following the AHREP, as well as significantly lower CK values at 24 hrs post. The AHREP significantly affected leukocyte counts, however, no treatment effects were observed. Greater isometric force was observed immediately post AHREP and at 24 hrs and 48 hrs with nucleotide supplementation. Nucleotide supplementation appears to attenuate muscle damage, HPA axis and immune system activation, and performance decrements following heavy resistance exercise.

Key Words: Weight training; muscle damage; HPA axis; myeloperoxidase; recovery, cortisol

INTRODUCTION

Nucleotides are essential to nearly all biological processes including DNA and RNA synthesis, coenzyme synthesis, energy metabolism, cellular signaling and protein homeostasis (8, 14, 17). Nucleotides produced by the body via *de novo* synthesis are often insufficient to meet the needs of rapidly proliferating tissues and therefore the salvage pathway is required to synthesize nucleotides from exogenous sources (12). As such, dietary nucleotides are necessary to maintain immune function, tissue growth and cellular repair (8, 12, 17). Exercise is a stressful and damaging stimulus, significantly increasing demands on the immune system and temporarily attenuating immune cell function. Consequently, there has been a growing interest in the potential implications of exogenous nucleotide supplementation on exercise-induced immune responses.

Recently, nucleotide supplementation demonstrated the ability to attenuate the transient immunosuppression that occurs following exercise (24, 25, 29, 30). Following acute exercise bouts, serum and salivary immunoglobulin levels were greater with nucleotide supplementation. Ostojic et al. (25) also demonstrated enhanced natural killer cell (NKC) count and cytotoxicity, demonstrating that nucleotide supplementation can impact both the adaptive and innate immune systems. Enhanced innate immune system activity could aid recovery from exercise, as neutrophils contribute to the removal of debris from damaged tissue (35). The innate immune system can also

induce secondary muscle damage via the generation of reactive oxygen species, further attenuating athletic performance (2, 3, 10). Consequently, the impact of nucleotide supplementation on innate immune system activity is of interest to athletes and active individuals.

Aside from increases in salivary immunoglobulins, McNaughton et al. (24, 25) have observed a decreased cortisol response to exercise, which would partially explain the reduced immunosuppression. Animal models have observed similarly attenuated cortisol responses to stressful stimuli (31, 34). Given the roles of cortisol in gluconeogenesis and glycogenolysis, a reduced cortisol response may indicate a reduction in the metabolic stress of the exercise bout as a result of nucleotide supplementation. In the days following stressful exercise, elevated cortisol levels could impair recovery, as cortisol can increase protein degradation and inhibit protein synthesis (18, 22). Unfortunately, McNaughton et al. (24, 25) did not analyze cortisol values beyond the immediate post-exercise time point.

Previously, Ostojic et al. (29, 30) and McNaughton et al. (24, 25) demonstrated the effects of nucleotide supplementation following acute exercise, but not the effects on recovery in the days following. Additionally, the previous investigations employed cycling and running exercise which is quite different in its recruitment and metabolic demands when compared to heavy resistance exercise. The effects of nucleotide supplementation on the response patterns to resistance exercise are currently unknown. Therefore, the primary purpose of this investigation was to determine

whether nucleotide supplementation affects the acute hypothalamic-pituitary axis (HPA) and immune response to resistance exercise. Additionally, we sought to determine if nucleotide supplementation could improve physical performance capabilities during recovery from such strenuous exercise.

METHODS

Experimental Approach to the Problem

To evaluate the effects of a dietary nucleotide supplement on resistance exercise stress and recovery, a double-blinded, placebo-controlled, within-group crossover design was used. Each subject completed a familiarization visit followed by two supplementation and testing cycles, which were separated by a one-week washout period. The two treatment cycles consisted of a nucleotide or placebo supplement, with cycle order randomized and balanced. Each cycle began with a two week loading phase in which subjects took the supplement while maintaining their normal exercise routines. At the beginning of the third week, an acute heavy resistance exercise protocol (AHREP) was completed. On this day, blood draws were taken before (PRE), immediately after (IP), and at 15, 30, 60 and 120 minutes after the AHREP. Blood samples were analyzed for changes in markers of stress, muscle damage, and immune system activation. Performance measures were taken before and after the AHREP to examine changes in peak force and power. To assess effects on recovery, subjects reported to the laboratory 24, 48 and 72 hrs following the AHREP for additional blood draws and performance testing.

Subjects

Ten men (mean ± SD; age = 23.6 ± 4.5 years; height =176.3 ± 5.5 cm; weight = 86.0 ± 12.7 kg) and ten women (mean ± SD; age = 22.2 ± 2.3 years; height = 160.4 ± 4.7 cm; weight = 62.0 ± 8.5 kg) completed the protocol. All subjects were resistance trained with a smith machine back squat one repetition maximum (1RM) of at least 150% of their body weight and were cleared by a physician for any musculoskeletal or pathological conditions which could affect the results of the investigation. All subjects completed a written informed consent prior to participation after having the benefits and risks of the investigation explained to them. The University of Connecticut Institutional Review Board for use of human subjects approved this investigation.

Procedures

Supplement Protocol

The nuBound® (Nu Science Laboratories, Inc., Boston, MA) supplement contains dietary nucleotides, which are extracted from yeast (saccharomyces cerevisiae). During the supplement treatment cycle, subjects took two capsules of nuBound® daily, one upon waking, and one following exercise. The two capsules (1000mg) contained 278mg of dietary nucleotides, 375mg amino acids (l-glutamine, l-methionine, l-lysine), riboflavin (4.5mg), folate (400mcg), biotin (188mg) and pantothenic acid (12mcg). Other ingredients included fructo-oligosaccharides (chicory root), inositol and sodium citrate.

During the placebo cycle, subjects followed a dosing schedule identical to the supplement cycle. The placebo capsules were identical in size, shape and color to the nucleotide supplement but contained only lactose and magnesium stearate. During the first treatment cycle, subjects recorded their daily dietary intake on a diet log. The log was then used to help subjects replicate their diet during the second treatment cycle. Subjects also replicated their activity protocol during the study for each cycle.

Subject Familiarization

Prior to beginning the supplement or placebo cycles, all subjects reported to the laboratory for familiarization with the study procedures. Proper technique for the warm up procedures, the isometric squat and countermovement jumps were demonstrated for the subjects, which they then practiced. Subsequently, a smith machine back squat one repetition maximum (1RM) was determined. Briefly, subjects performed eight to ten repetitions at 50%, followed by three to five repetitions at 85% of his or her estimated 1RM. Two to four maximal attempts were then performed to determine the individual's 1RM. Following 1RM testing, subjects performed a full AHREP as described below, starting with a load equal to 75% of the previously determined 1RM.

AHREP Visit

On the day of the AHREP, subjects reported to the laboratory between 0530 and 0930, following a 12 hour fast. Starting times for the AHREP were matched between cycles. Subject hydration was assessed via urine specific gravity using a handheld refractometer (Reichert, Lincolnshire, IL) with a USG ≤ 1.025 considered adequate. An

indwelling cannula was inserted into a superficial forearm vein of the subjects, and the PRE blood draw taken 10 minutes after cannula insertion. Next, subjects completed a standardized warm up procedure consisting of five minutes on a cycle ergometer and a series of dynamic stretches. After the warm up, subjects completed the pre-AHREP performance tests followed by the AHREP, as detailed below. Immediately after the AHREP the IP blood draw was obtained, and post-AHREP performance tests were conducted. Subjects remained in the laboratory for two hours to allow for blood collection at 15, 30, 60 and 120 minutes post-AHREP.

Recovery Visits

At 24, 48 and 72 hrs after the completion of the AHREP, subjects reported to the laboratory for recovery testing. Adequate hydration was confirmed, and subjects then sat for 10 minutes before a single blood draw. Subjects then performed an isometric squat MVC and a set of three maximal effort countermovement jumps.

Acute Heavy Resistance Exercise Protocol (AHREP)

The AHREP consisted of six sets of 10 repetitions of back squats to parallel on a Smith machine, allowing only vertical translation of the bar. The starting weight was approximately 75% of the 1RM, as determined on the familiarization day. If a subject was unable to complete all 10 repetitions, the weight was reduced at the discretion of the testers, and the remaining repetitions were completed. The weight was reduced to allow for completion of the required repetitions, with the goal of achieving the highest possible load volume. After the first and second set, two minutes of rest were given;

three minutes were given after each of the remaining sets as pilot testing indicated that the additional rest allowed subjects to complete the AHREP with a higher load.

Performance Measures

Peak force was obtained during a maximal isometric squat, and peak power was obtained during a series of three maximal effort countermovement jumps. The maximal isometric squat was performed on a Smith machine, with force production measured and analyzed via force plate (Fitness Technology 400 series performance force plate, Australia) and Ballistic Measurement System software (Software Version 2009.0.0). The height of the Smith machine bar was adjusted so that subjects were positioned with an approximate 135° knee joint angle. Subjects were instructed to push into the bar as if they were performing a squat, gradually increasing force until they reached maximal effort, holding the maximal effort, and then a slow reduction of force to resting levels (a trapezoidal force development curve). The total length of time for the isometric squat test was 10 seconds. Countermovement jumps were also performed on a force plate. Subjects kept their hands on their hips and performed three consecutive maximal-effort countermovement jumps.

Blood Collection

On the day of the AHREP, blood was collected at PRE, IP, and 15, 30, 60 and 120 minutes post-AHREP via an indwelling cannula kept patent via sterile saline. Prior to each blood draw, 3 mL of fluid was extracted and discarded. Single blood draws were taken at 24, 48 and 72 hours post AHREP. Whole blood was collected and placed in

serum tubes or plasma tubes. The samples were then centrifuged, aliquoted, and stored at -80° C until subsequent analyses.

Biochemical Analyses

The creatine kinase-SL assay (SEKISUI, Charlottetown, Canada) was performed in duplicate using serum samples. A Thermo Scientific Biomate3 Spectrophotometer (Pittsburgh, PA) was used to read the assays at a wavelength of 340 nm. The coefficient of variation (CV) was 4.2%. Lactate was measured in EDTA-plasma samples using a liquid lactate reagent (Point Scientific, Canton, MI) and assayed according to Gutmann et al. and Noll et al. (15, 27). Serum Uric acid was measured utilizing uric acid reagents purchased from Pointe Scientific (Canton, MI) and performed according to manufacturer's instructions. Serum cortisol was measured using an ELISA (CALBiotech, Spring Valley, CA), with a sensitivity of 11.1 $nmol \bullet L^{-1}$. Myeloperoxidase (MPO) was measured in EDTA-plasma samples using an ELISA (ALPCO, NH) with a sensitivity of 1.08 $ng \bullet mL^{-1}$. Lactate, uric acid, cortisol and MPO were analyzed in duplicate on a Versamax tunable microplate reader (Molecular Devices, Sunnyvale, CA) at the appropriate wavelength for the given assay. Intra and inter-assay CVs for lactate, uric acid, cortisol and MPO were below 3.9%, 4.7%, 7.2% and 6.3%, respectively.

Whole blood was analyzed for absolute neutrophil, lymphocyte and, monocyte, eosinophil and basophil counts as part of a complete blood count with differential performed by Quest Diagnostics (Madison, NJ) using an automated hematology analyzer.

Statistical Analyses

Data are presented as means ± SD. Normality and homogeneity of variance were confirmed for the selected dependent variables. Data were analyzed using one between (sex) by two within (treatment, time point) mixed methods analyses of variance. When significant differences were detected, Fisher's least significant difference (LSD) post hoc analysis was performed to make pairwise comparisons. Statistical significance was set at $p \leq 0.05$.

RESULTS

The primary finding of this investigation was that nucleotide supplementation significantly altered resistance exercise-induced changes in cortisol, MPO, and CK concentrations, as well as isometric force production. As expected, the stress of the AHREP perturbed all variables measured. Sex differences were observed in terms of lactate, MPO, CK, isometric force, and CMJ power.

The AHREP induced significant acute increases in cortisol values in male and female placebo groups, which returned to baseline by 60 and 30 minutes post-AHREP, respectively. In both sexes, nucleotide supplementation resulted in significantly lower

cortisol values at IP, +15m and +30m when compared with the corresponding time points under placebo. No sex-specific differences were observed. Cortisol values are presented in Figure 1.

<Insert Figure 1 here>

Following the AHREP, MPO increases were observed in both sexes under both treatment conditions. Additionally, men demonstrated elevated MPO levels at 72 hours post-AHREP under the placebo treatment only. Acutely, nucleotide supplementation resulted in significantly reduced MPO levels following the AHREP and during recovery time points in both men and women. Women demonstrated reduced MPO values at rest after nucleotide supplementation. MPO values are presented in Figure 2.

<Insert Figure 2 here>

Acute elevations in lactate were observed following the AHREP under both treatment conditions in both sexes. All groups demonstrated elevations at IP, +15m, +30m, and +60m, but returned to baseline values by +120m. Nucleotide supplementation had no distinct effect on lactate values in men or women. When sexes were compared, women demonstrated significantly lower lactate values at IP in both treatments. Lactate values are presented in Figure 3.

<Insert Figure 3 here>

During the recovery period, CK values were significantly elevated above baseline values in men in women, regardless of treatment condition. CK values at 24 hours post-

AHREP were significantly lower in nucleotide-supplemented groups when compared with placebo treatment. Sex differences in CK were observed at baseline and recovery in the nucleotide-supplemented groups, but only during recovery in the placebo groups. CK values are presented in Figure 4.

<Insert Figure 4 here>

Significant uric acid increases were observed in men under both treatments at +60m and +120m, however, no significant changes were observed in women. When compared to men, women demonstrated significantly lower uric acid values at all time points. No significant effects for nucleotide supplementation were observed in either sex. Uric acid values are presented in Figure 5.

<Insert Figure 5 here>

A similar post-AHREP increase in neutrophil count was observed after both treatments in men and women. Lymphocytes also exhibited a significant time effect, where all groups experienced elevated counts at IP, and a subsequent decrease below baseline within 30 minutes. Monocytes were elevated at IP regardless of treatment or sex. Men and women in the placebo treatment demonstrated a decrease in the monocytes below baseline values during the acute post-AHREP period, but this treatment effect was not statistically significant. Women exhibited significantly lower monocyte counts at IP in both treatments, and at 48 hours post-AHREP in the placebo condition. The examined leukocyte populations did not appear to respond to nucleotide supplementation. Absolute leukocyte counts are shown in Tables 1 and 2.

<Insert Tables 1 & 2 here>

The AHREP had a significant detrimental effect on isometric force generation in men and women under placebo treatment. Isometric force generation returned to baseline values in nucleotide supplemented women within 24 hours of the AHREP, however, men and women in the placebo treatment required 48 hours for fully recovery. Men receiving the nucleotide supplementation demonstrated no impairment of isometric force immediately after the protocol or during the recovery days. When compared to men, women demonstrated lower isometric force values at all time points for both treatments. Isometric force values are presented in Figure 6.

<Insert Figure 6 here>

Decrements in countermovement jump peak power were observed immediately following the AHREP in all subjects, however, values returned to baseline within 24 hours. No effects of nucleotide supplementation were observed. Similar to isometric force, women demonstrated lower peak power values at all times and treatments when compared to men. Countermovement jump peak power values are presented in Figure 7.

<Insert Figure 7 here>

DISCUSSION

The primary finding of this investigation is that a dietary nucleotide supplement reduced markers of HPA and inflammatory activity, and these changes corresponded with reductions in tissue damage and the preservation of force production capabilities. Sex-specific differences in the response to nucleotide supplementation included the absence of improvement of physical recovery in women, who tended to demonstrate smaller inflammatory activity (lower MPO), and lower lactate, CK, and uric acid generally. These sexual dimorphic observations indicate that nucleotide supplementation produced modest positive effects primarily in men, who might have differed from women in terms of absolute exertion, normal or stress-related nucleotide metabolism, or immune/inflammatory responses to heavy resistance exercise.

Acute resistance exercise consistently results in transient increases in cortisol values (20, 21, 33). In accordance with previous findings, post-AHREP increases in cortisol were observed in both men and women with placebo. In contrast, no post-AHREP cortisol increases were observed in the male or female nucleotide groups. Nucleotide supplementation has previously been demonstrated to elicit similar reductions in the cortisol response following aerobic exercise (24, 25). Furthermore, nucleotide supplementation has been shown to attenuate the cortisol response to a variety of stressful stimuli in multiple species (31, 34). The mechanism(s) through which nucleotides regulate the cortisol response is currently unknown, however, may be due to the ability of adenosine and uracil to increase hepatic glucose output (14, 16). During exercise, low blood glucose stimulates cortisol release to increase glycogenolysis. If nucleotide supplementation increased hepatic glucose output, blood glucose levels may

have been better preserved during the AHREP, thus attenuating a key signal for cortisol secretion.

The oxidative enzyme, myeloperoxidase (MPO), is released from activated neutrophils and serves as an indirect measure of neutrophil activation and innate immune system activity. As in previous aerobic exercise investigations, MPO increased acutely following the AHREP (5-7, 11). Though acute increases were observed in both placebo and nucleotide supplemented groups, MPO values were significantly lower after nucleotide supplementation. Additionally, decreased pre-AHREP MPO values were observed in women. Glucocorticoids, such as cortisol, have been suggested to stimulate neutrophil degranulation, increasing serum concentrations of oxidative enzymes including MPO (26). Therefore the attenuated MPO increase observed with nucleotide supplementation might reflect reduced innate immune activation subsequent to a reduced cortisol response.

While beyond the scope of this investigation, from a physiological viewpoint, we suggest that a potential attenuation of endotoxemia could explain the observed differences in the cortisol and MPO response. During strenuous exercise, slight ischemia in the gut can damage mucosa and disrupt the mucosal barrier, resulting in increased permeability, and the resultant passage of pathogens, such as lipopolysaccharide, into the circulation (4, 23). A potent inflammatory agent, LPS can stimulate the activation of neutrophils, production of inflammatory cytokines, and an HPA response that would include increased glucocorticoid secretion (13, 32, 36).

Animal models have demonstrated the ability of dietary nucleotide intake to promote recovery of damaged gut mucosa (28) and to improve mucosal barrier function (1, 19). Therefore, the nucleotide supplementation may have reduced or prevented the influx of inflammatory pathogens into circulation, thus attenuating the cortisol and MPO response.

The AHREP induced significant muscle damage in all groups. Though elevated above baseline, CK values at were significantly lower in the nucleotide-supplemented groups at the 24-hour post-AHREP time point. Reduced CK values at 24 hours post-AHREP but not 48 or 72 hours post-AHREP suggests that nucleotide supplement reduced exercise-induced muscle damage, rather than improving recovery. Generally, because neutrophil activation is associated with the production of reactive oxygen species (9, 11), which promote secondary (inflammation-induced) muscle damage, reductions in MPO may partially explain improvements in muscle damage. Previously, McNaughton et al. (24) reported an absence of effect of nucleotide supplementation on post-exercise CK values, though differences in the timing of assessment likely explains the conflicting results. In the current investigation, CK measurements were extended to 72 hours, whereas McNaughton et al. (24) measured CK immediately after exercise only.

In accordance with the attenuated cortisol response, neutrophil activation, and muscle damage, nucleotide supplementation attenuated performance decrements. After nucleotide supplementation, men produced greater isometric force immediately

after the AHREP and then 24 and 48 hours later. Women also demonstrated greater isometric force, but only 24 hours after exercise. As structural muscle damage can impair force generation (3, 9), decreased muscle damage sustained by nucleotide-supplemented subjects likely explains these observations.

Regardless of sex or treatment, all leukocyte populations were affected by the AHREP. The lack of differences was surprising, given the differences in immune function, as well as previous investigations. Our findings expand upon the findings of Ostojic et al. (29) who reported no difference in total leukocyte count as a result of nucleotide supplementation. Together these findings suggest that the effects of nucleotide supplementation on immune cell activity are independent of changes in absolute leukocyte counts. Alternatively, offsetting changes in immune cell phenotypes are possible, but would require additional immunological phenotyping beyond the scope of the present investigation. However, this represents a promising area in immunology that may have important implications for our understanding of responses and adaptations to resistance exercise and deserved further investigation.

The results of this investigation in general support the previous studies in that nucleotide supplementation may also attenuate the stress response, reduce muscle damage, and preserve force production capabilities following intense resistance exercise. Together, these effects could improve recovery from strenuous exercise. Despite these promising findings, more research is needed to determine the mechanisms through which nucleotide supplementation exerts its impact.

PRACTICAL APPLICATIONS

Dietary nucleotide supplementation reduces the stress response to resistance exercise as evidenced by the attenuated cortisol and MPO values. Furthermore, dietary nucleotide supplementation appears to reduce resistance exercise-induced muscle damage, resulting in a greater preservation of force production capability.

Acknowledgements

This study was supported in part by a grant from Nu Science Labs, Inc., Boston, MA, and the makers of the nuBound nucleotide supplement. We would like to thank all of the subjects who volunteered for this study and worked so hard to make it all work. We also thank our medical monitor Dr Jeffrey Anderson. Current address: LT Brian Kupchak, Ph.D., MSC, USN, Deputy Director of Research, Consortium for Health and Military Performance (CHAMP) *Center of Excellence* Uniformed Services University, Bethesda, MD 20877

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Figure Legends

Figure 1. Data are presented as means ± SD. Nucleotide supplementation attenuated the cortisol response to resistance exercise in both men in women. Post resistance exercise increases in cortisol concentrations were only observed in the placebo conditions. * Significantly different from corresponding placebo condition. # = significantly different from corresponding PRE value. Significance set at $p \leq 0.05$.

Figure 2. Data are presented as means ± SD. Nucleotide supplementation attenuated the myeloperoxidase response to resistance exercise in both men in women. Post resistance exercise increases in MPO concentrations were only observed in the both nucleotide and placebo conditions. A sex difference in MPO concentrations was observed only in the nucleotide condition. * Significantly different from corresponding placebo condition. # = significantly different from corresponding PRE value. † = significantly different from men in the corresponding condition. Significance set at $p \leq 0.05$.

Figure 3. Data are presented as means ± SD. No effect for nucleotide condition was observed for lactate concentrations in either sex. Heavy resistance exercise induced a significant increase in lactate in all groups. A between sex difference in lactate was observed at IP in both nucleotide and placebo conditions. # = significantly different from corresponding PRE value. † = significantly different from men in the corresponding condition. Significance set at $p \leq 0.05$.

Figure 4. Data are presented as means ± SD. Nucleotide supplementation attenuated the cortisol increase at 24 hours post resistance exercise in both men and women.

Heavy resistance exercise induced a significant increase in creatine kinase in the all groups. In both nucleotide and placebo conditions, women demonstrated reduced creatine kinase concentrations. * Significantly different from corresponding placebo condition. # = significantly different from corresponding PRE value. † = significantly different from men in the corresponding condition. Significance set at $p \leq 0.05$.

Figure 5. Data are presented as means ± SD. No effect for nucleotide supplementation was observed for uric acid in either sex. Men demonstrated an increase in uric acid at 60 and 120 minutes post resistance exercise in both conditions. No increase in uric acid was observed in women at any time point in either condition. At every time point measured, women demonstrated significantly reduced uric acid concentrations when compared with men in the corresponding condition. # = significantly different from corresponding pre value. † = significantly different from men in the corresponding condition. Significance set at $p \leq 0.05$.

Figure 6. Data are presented as means ± SD. Nucleotide supplementation attenuated the decrease in isometric force following the resistance exercise protocol in both men and women. In nucleotide-supplemented men, no decrease in isometric force was observed. Women demonstrated reduced isometric force when compared with men in the corresponding treatment at every time point. * = Significantly different from corresponding placebo condition. # = significantly different from corresponding pre value. † = significantly different from men in the corresponding condition. Significance set at $p \leq 0.05$.

Figure 7. Data are presented as means ± SD. Nucleotide supplementation had no effect on countermovement jump peak power in either sex. The heavy resistance exercise protocol attenuated peak power in three of the four groups at the IP time point. Women demonstrated reduced isometric force when compared with men in the corresponding treatment at every time point. * = significantly different from corresponding placebo condition. # = significantly different from corresponding pre

value. † = significantly different from men in the corresponding condition. Significance set at $p \leq 0.05$.

Table 1. Average Immune Cell Counts After Nucleotide Supplementation in Men

	Neutrophils		Lymphocytes		Monocytes	
Time	Nucleotide	Placebo	Nucleotide	Placebo	Nucleotide	Placebo
Pre	3057 ± 827	3353 ± 1674	2033 ± 502	1996 ± 624	458 ± 89	478 ± 126
IP	4821 ± 1339*	5548 ± 2739*	4528 ± 1246*	4108 ± 1337*	922 ± 209*	1001 ± 317*
15	4134 ± 1231*	4479 ± 1991*	2918 ± 967*	2635 ± 1040*	585 ± 163	647 ± 225*
30	3449 ± 1112	3898 ± 1693*	1688 ± 553*	1540 ± 483*	418 ± 92	451 ± 112
60	3457 ± 1884	4138 ± 1990*	1253 ± 310*	1194 ± 303*	384 ± 87	414 ± 106*
120	5475 ± 2988*	5512 ± 2877*	1250 ± 259*	1261 ± 312*	421 ± 81	494 ± 132
24 h	3337 ± 714	3978 ± 2158	1739 ± 725	1645 ± 550	472 ± 107	485 ± 144
48 h	3023 ± 459	3497 ± 1592	1902 ± 588	1813 ± 529	472 ± 107	545 ± 168
72 h	3328 ± 953	3336 ± 1792	1929 ± 663	1687 ± 363	527 ± 138	551 ± 132*

* Significantly different from corresponding Pre value ($P < 0.05$)
‡ Significantly different from corresponding value in men

Table 2. Average Immune Cell Counts after Nucleotide Supplementation in Women

	Neutrophils		Lymphocytes		Monocytes	
Time	Nucleotide	Placebo	Nucleotide	Placebo	Nucleotide	Placebo
Pre	3364 ± 1985	3576 ± 2681	2337 ± 753	2822 ± 962	482 ± 235	550 ± 473
IP	5636 ± 3631*	4626 ± 1635*	3898 ± 828*	3887 ± 742*	681 ± 256*‡	720 ± 195*‡
15	4417 ± 2814*	3766 ± 1771	2063 ± 527‡	2458 ± 330	491 ± 308	467 ± 194
30	4027 ± 2485*	3485 ± 1932	1498 ± 401*	1759 ± 349*	362 ± 116	401 ± 170*
60	4310 ± 2431*	4348 ± 1890*	1366 ± 348*	1640 ± 401*	383 ± 208	399 ± 160*
120	5440 ± 2553*	5399 ± 1905*	1454 ± 439*	1625 ± 340*	440 ± 262	407 ± 174*
24 h	2714 ± 1044	3333 ± 1426	2105 ± 594	2351 ± 683	445 ± 297	443 ± 117
48 h	3210 ± 1609	3508 ± 1343	2122 ± 628	2327 ± 554	476 ± 287	429 ± 134‡
72 h	3083 ± 1882	3134 ± 1426	2000 ± 525	2270 ± 596	466 ± 344	442 ± 151

* Significantly different from corresponding Pre value ($P < 0.05$)
‡ Significantly different from corresponding value in men